8-1998

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Ingestion of arsenic-contaminated drinking water is associated with an increased risk of several cancers, including skin and bladder malignancies; but it is not yet clear whether such adverse effects are present at levels to which the U.S. population is exposed. In New Hampshire, detectable levels of arsenic have been reported in drinking water supplies throughout the state. Therefore, we have begun a population-based epidemiologic case–control study in which residents of New Hampshire diagnosed with primary squamous cell ($n = 900$) and basal cell ($n = 1200$) skin cancers are being selected from a special statewide skin cancer incidence survey; patients diagnosed with primary bladder cancers ($n = 450$) are being identified through the New Hampshire State Cancer Registry. Exposure histories of these patients will be compared to a control group of individuals randomly selected from population lists ($n = 1200$). Along with a detailed personal interview, arsenic and other trace elements are being measured in toenail clipping samples using instrumental neutron activation analysis. Household water samples are being tested on selected participants using a hydride generation technique with high-resolution inductively coupled plasma mass spectrometry. In the first 793 households tested, arsenic concentrations ranged from undetectable (0.01 µg/l) to 180 µg/l. Over 10% of the private wells contained levels above 10 µg/l and 2.5% were above 50 µg/l. Based on our projected sample size, we expect at least 80% power to detect a 2-fold risk of basal cell or squamous cell skin cancer or bladder cancer among individuals with the highest 5% toenail concentrations of arsenic. — *Environ Health Perspect* 106(Suppl 4):1047–1050 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/1047-1050karagas/abstract.html*

Key words: epidemiologic studies, case–control studies, nonmelanoma skin cancer, squamous cell skin cancer, basal cell skin cancer, bladder cancer, arsenic, heavy metals, exposure assessment

Introduction

\Arsenic is one of the eight metals and 22 agents listed by the Office of Solid Waste and Emergency of the U.S. Environmental Protection Agency (U.S. EPA) as a concern at Superfund sites and is naturally present in a variety of ores and minerals. Humans are exposed to arsenic primarily through food, and in some regions, through drinking water. Arsenic also has been used for various industrial purposes such as pesticides, wood preservatives, feed additives, and semiconductor applications (e.g., gallium arsenide). Patients were treated with arsenic-containing drugs such as potassium arsenite (e.g., Fowler solution) for benign skin conditions in the 19th and early 20th century, but arsenic is now rarely used medicinally (*1*).

In high concentrations (e.g., > 2000 µg/kg/day), arsenic is toxic to humans, and among the reported long-term health effects associated with nonfatal doses are vascular diseases, diabetes, and cancers—specifically cancers of the skin (basal cell and squamous cell), lung, bladder, kidney, and liver. These observations have been made among individuals exposed to arsenic occupationally or through drinking water contamination or pharmacologic uses (*2*). In humans, organic arsenic compounds (e.g., arsenobetaine, found in seafood) are not as toxic as inorganic arsenic. The carcinogenic effects of organic arsenic are uncertain, although there was no apparent increase in skin cancers among patients treated with organic arsenics for syphillis (*3*).

Estimates of the dose–response relationship between drinking water arsenic exposure and cancer have been made from studies conducted in a region of Taiwan that had artesian well water highly contaminated with arsenic. In a well-known household prevalence survey of skin cancer (*4*) published about 30 years ago, villages with median water arsenic concentrations of 170, 470, and 800 µg/l had skin cancer prevalence rates of 26, 101, and 214 per 1000 persons, respectively (*4*). More recently, Brown and colleagues (*5*) performed a reanalysis of these data using a multistaged model. A model that included both a linear and quadratic term in dose improved the fit only slightly; thus there was no definitive conclusion regarding the actual shape of the dose–response curve. Among the limitations of the Taiwanese study are that data are provided for the median concentration for all wells in a village, and in some villages concentrations varied considerably, i.e., from nearly 0 to over 1200 µg/l. Of some reassurance is that data from a study conducted in Mexico (with similar limitations) (*5*) also appeared to fit the model derived from Taiwan data. Smith and colleagues (*6*) modeled standardized mortality ratios of bladder, liver, lung, and kidney malignancies computed for the endemic region of Taiwan using rates for the country at large as the standard. A linear dose–response relationship was suggested by their analysis.

This paper is based on a presentation at the Symposium on the Superfund Basic Research Program: A Decade of Improving Health through Multi-Disciplinary Research held 23–26 February 1997 in Chapel Hill, North Carolina. Manuscript received at *EHP* 11 December 1997; accepted 22 April 1998.

We are indebted to the inspirational leadership and scientific insights provided by the late K. Wetterhahn. We gratefully acknowledge the collaboration of E.R. Greenberg, S.K. Spencer, T. Stukel, J. Heaney, and A. Schned, and the ongoing advice of J. Hamilton. We also appreciate the important contributions of study staff, research coordinator, V. Stannard, the statistical analysts J. Weiss and L. Mott, and the many physicians who comprise the New Hampshire Skin Cancer Study Group and have made the study possible. Funding for the study was provided by the National Institutes of Health grants NIEHS ES-07373, NCI CA57494, and NCI CA61108.

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Abbreviations used: BCC, basal cell carcinoma; INAA, instrumental neutron activation analysis; HR-ICP-MS, high-resolution inductively coupled mass spectrometer; SCC, squamous cell carcinoma; U.S. EPA, U.S. Environmental Protection Agency.

The risk models derived from the Taiwanese data indicate that concentrations below 50 µg/l (the current maximum contaminant level for drinking water in the United States) may be carcinogenic. Consequently, there is considerable debate about what levels of arsenic are safe. Part of the difficulty is that we have limited data for the United States at levels found in the United States. The assessment of cancer risk at concentrations below 170 µg/l required extrapolation (i.e., where actual data do not exist) and estimates have differed to some extent based on model assumptions (e.g., the point of intercept, consideration of arsenic exposure through food) and model choice (e.g., multistaged model or linear regression). Nonetheless, a small case–control study conducted in Utah found some evidence of an increased risk of bladder cancer in relation to drinking water arsenic, and in this population water concentrations of arsenic were primarily in the 10 to 50 µg/l range (*7*). Previous ecologic studies conducted in the United States have not found an association between water levels of arsenic and skin cancer occurrence (*8,9*). These results are difficult to interpret because each of the geographic areas studied had a broad range of water arsenic concentrations. Additionally, one of the U.S. studies (*9*) used mortality rates, which is a poor measure of occurrence for skin cancer.

Therefore, to help clarify the risk of cancer in relation to arsenic exposure in a geographically defined U.S. population, we began an epidemiologic case–control study of nonmelanoma skin cancers in 1993 and added bladder cancer to our investigation in 1995. Among our major objectives are to evaluate whether arsenic exposure is related to an increased risk of skin or bladder cancer in the New Hampshire population, to study methods of quantifying an individual's arsenic exposure, and to examine the sources and mechanisms of arsenic's presence in the drinking water supply.

Study Design

New Hampshire Study

New Hampshire is a rural state with a population of about 1.1 million (1990 U.S. Census) (*10*). Private water supplies serving fewer than 25 individuals or 15 households are not regulated as part of the U.S. EPA Safe Drinking Water Act (*11*) but an estimated one-third of the New Hampshire population uses private water. Arsenic is present in drinking water throughout the state, with levels as high as 600 µg/l detected though volunteer testing by the State of New Hampshire Water Supply and Pollution Control Commission; 5% of private wells are estimated to contain arsenic concentrations above 50 µg/l.

A population-based study of nonmelanoma skin cancer incidence is feasible in New Hampshire because of the unique availability of a statewide skin cancer registry. Nonmelanoma skin cancers are excluded from virtually all central cancer registries; therefore, epidemiologic studies of these malignancies are sparse in the United States and elsewhere. Yet nonmelanoma skin cancers are considered the most common cancers in humans (*12*). As part of our study, newly diagnosed basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) of the skin are being identified through a network of over 90% of practicing dermatologists and all pathology laboratories in New Hampshire and bordering areas. Routine visits are made to each physician's office and each pathology laboratory. Records are reviewed for basic demographic information, prior history of skin cancer, and histologic type and anatomic location of newly diagnosed BCCs and SCCs of the skin. Although *in situ* SCC (including Bowen's disease) is also associated with arsenic exposure (*13*), we were concerned that earlier staged lesions may be more susceptible to selection factors, i.e., people with known arsenic exposure may be more likely to seek medical attention or the physicians themselves more likely to biopsy and diagnose a lesion. Therefore, our case–control study includes only invasive SCC along with BCC.

We are randomly selecting a population-control group of a comparable age and sex distribution as the cancer cases from drivers' license records (for those less than 65 years of age) and from Medicare enrollment files (for those 65 years of age and older). Among the advantages of population-based control groups (vs clinic or hospitalized patients with other diagnoses) are that they are less prone to biases and also permit inference about exposure prevalence in the population at large (*14*).

We were particularly interested in bladder cancer in New Hampshire because maps of cancer mortality for state economic areas in the United States showed that bladder cancer mortality rates were in the highest 10% throughout the New England states, including New Hampshire (*15*). A case–control study conducted in Vermont and New Hampshire approximately 20 years ago found that occupational exposure in the textile and leather industries could explain only part of this excess (*15*). For our study, bladder cancers are ascertained from the New Hampshire State Cancer Registry, which operates a rapid reporting system. By state law, practitioners are required to provide an initial report of cancer within 15 days after diagnosis, and a definite report can follow within 120 days. To be eligible for our study, both skin and bladder cancer cases and controls must be residents of New Hampshire and 25 to 74 years of age. Because our study includes an individual biologic measure of arsenic exposure, we have not sought surrogate responses (e.g., from a spouse, close relative, or friend) for deceased individuals.

Personal Interview

Those who agree to take part in the study undergo an extensive in-person interview covering residence, occupation, medical history, lifestyle factors (e.g., use of tobacco), and family history of cancer. This enables us to control for potentially confounding factors and to evaluate whether other individual characteristics (e.g., smoking) modify the association between arsenic and skin or bladder cancer. Also, we examine whether other factors (e.g., occupational history) are contributors to arsenic exposure.

Participants are asked to document each place they have lived and to indicate the type of water supply (private vs public) for each residence. Interview questions relating to their household water supply include the type of water supply that serves their household (e.g., public water, shared well, private well, or spring); the duration of use; type of supply (for private water supplies; e.g., artesian, shallow, or spring); how many glasses of water per day are consumed in foods or beverages from this drinking water supply; and use of water filters (e.g., what type and for how long).

Arsenic Determinations

The latency period for arsenic and cancer occurrence is not yet known, but is likely several years if not decades. For this reason, we sought a long-term individual measure of exposure. Arsenic is rapidly cleared from the blood and excreted in urine. Therefore, arsenic concentration in these fluids will reflect relatively recent exposure (e.g., in the past several hours or a few days). Specific forms of arsenic can be analyzed in urine (e.g., inorganic and various methylated forms), and the fraction of these forms in urine may reflect individual differences in

the ability to metabolize arsenic (*16*). Arsenic accumulates in hair and nail tissue by binding to sulfhydryl groups of keratin, and for this reason, these tissues have been used to trace arsenic poisoning. Toenail clippings are routinely used in epidemiologic studies because they are relatively easy to collect and are less susceptible to external contamination than hair. Toenails provide a larger sample than fingernails and take longer to grow. In a sample of nurses tested twice for arsenic and other trace elements 6 years apart, arsenic was among the most highly correlated of the elements they analyzed (a correlation of greater than 0.5) (*17*). Measurement of arsenic directly in water samples is another alternative. However, at the time our study began, we were uncertain whether water concentrations varied appreciability over time, i.e., seasonally or from year to year. Moreover, water is only one potential source of exposure. In our small pilot study, the overall correlation between water and toenail concentrations of arsenic was 0.67 (*18*); however, half of the samples were drawn from regions of New Hampshire known to have relatively high water levels of arsenic. A significant correlation between water concentrations and both hair and toenail levels of arsenic was also recently reported by Chiou and colleagues (*19*). In this study, genetic polymorphism in the *GSTM1* or *GSTT1* loci, which had an effect on the metabolic forms of arsenic in urine, did not affect nail or hair concentrations.

For our case–control study, toenail clipping samples are being analyzed for arsenic and other trace elements by instrumental neutron activation analysis (INAA) at the University of Missouri Research Reactor (Columbia, MO) using a standard comparison approach as described previously (*20*). This method reduces and possibly eliminates external contamination. Matrix-matched quality control samples, having known arsenic content, and analytical blanks are analyzed with the samples and standards. The detection limit for arsenic measured by INAA is approximately 1 µg/l. An additional advantage to INAA is that samples can be analyzed for arsenic along with other trace elements hypothesized in experimental studies to interact with arsenic carcinogenesis (*21*) (e.g., selenium, zinc).

Water samples are being tested using a hydride-generation technique with a high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) (*22*). Using this approach, a quantitative estimate of arsenic is obtained for 99% of the water samples. The instrument is a Finnigan MAT Element (Bremen, Germany) HR-ICP-MS. Although lower detection limits are possible, the quantification limit for samples was set to 0.01 µg/l (0.001 µg/l for blanks) to avoid excessive washout times. Levels below 1 µg/l are not suspected of posing a health risk; therefore, from a practical perspective it may not be necessary to measure arsenic at such trace levels. However, we also are interested in the correlation between toenail and water concentrations and arsenic is detectable in all nail samples. Likewise, we are examining the geographic distribution of arsenic in water throughout the state.

Accurate quantification of arsenic necessitates strict precautions against contamination. All sample preparations and analyses are carried out in a trace-metal clean HEPA-filtered-air environment. Commercially washed (mineral-free) high-density polyethylene bottles meet U.S. EPA standards for water collection (I-Chem, Newcastle, DE); however, we are unaware of any published data on the performance of these bottles for arsenic determination. Therefore, we prepared acid-washed polyethylene bottles in our class 100 clean room and initially used these on all households with private wells and on a random sample of those with public water. Commercial bottles were used for the rest of the households because we expected a smaller fraction of public water would have detectable arsenic. Powderless latex gloves are worn during the collection and bottles are kept in a washed sealed plastic bag. Samples of cold water are taken after running the tap for at least 1 min to avoid metal precipitation in the pipes. Samples are immediately placed back into the sealed bag. Duplicate samples are drawn on 10% of the households (every 10th interview) throughout the study, and field blanks are performed quarterly by each interviewer. To compare the performance of the two bottle-washing methods, we tested water from a sample of households using both types. All bottles are labeled with identification numbers that do not reveal the case–control status of the study participants or whether the sample was a replicate from the same household. In addition to these quality control samples, analytical blanks and potential instrumental drifts are carefully monitored, and instrument standardization and reproducibility is performed with certified standard reference materials.

Water Arsenic Results

In the 793 households tested to date (Table 1), 41% reported using a private well or spring (serving 15 or fewer households or less than 25 individuals). Arsenic concentrations range from undetectable (< 0.01 µg/l) to 180 µg/l. Over 25% of the private wells contained more than 2 µg/l of arsenic, over 10% were above 10 µg/l, and 2.5% were over 50 µg/l. In a preliminary analysis of the population controls, we found that participants residing in the three major cities of New Hampshire (Concord, Manchester, or Nashua) had lower water arsenic on average (data not shown). However, participants from these areas comprise only about 20% of our sample.

A preliminary analysis was performed on the replicate samples tested thus far. Simple random effects models were used to calculate intraclass correlations for the log transformed arsenic measurements (*23*). The intraclass correlation coefficient for replicate samples was 0.98 and did not appear to differ by bottle type (i.e., laboratory cleaned or commercially cleaned containers). Given this high correlation, we concluded that the bottle types yield highly comparable and reliable results in our laboratory.

Table 1. Preliminary data on water arsenic concentrations among New Hampshire study participants.

	Type of water supply		
Water arsenic, µg/l	Private, %[a]	Public, %[b]	Total, %[c]
< 0.01	0.9	0.4	0.6
0.01–0.10	27.8	30.7	29.5
0.11–0.50	23.5	52.2	40.5
0.51–1.00	12.3	8.5	10.1
1.01–2.00	7.7	3.4	5.2
2.01–10.00	15.4	3.6	8.4
10.01–50.00	9.9	1.1	4.7
> 50.00	2.5	0.0	1.0
Total, all samples	100.0	100.0	100.0

[a]Households tested = 324; range < 0.01–180.09; median 0.42. [b]Households tested = 469; range < 0.01–49.53; median 0.17. [c]Households tested = 793; range < 0.01–180.09; median 0.20.

Sample Size and Study Power

Based on existing risk assessment models, we expect that the relative risks of cancer associated with low levels of arsenic exposure may be relatively small (i.e., on the order of 1.5–2.0). We expect to interview about 1200 BCC cases, 900 SCC cases, 450 bladder cancer cases, and 1200 controls. With this sample size, we anticipate 80% power to detect an odds ratio of 1.6, 1.7, and 1.9, respectively, for BCC, SCC, and bladder cancer among individuals with the highest 5% of toenail arsenic concentrations with a significance level (alpha) of 0.05. At an alpha of 0.10, the minimum detectable odds ratios are 1.5, 1.6, and 1.8 for BCC, SCC, and bladder cancer. The minimum detectable odds ratios for the highest 2% of arsenic concentrations are about 2.0 for BCC and SCC and 2.5 for bladder cancer, with an alpha of 0.05, and 1.9 and 2.3, respectively, with an alpha of 0.10. We will increase our study power by analyzing continuous variables (i.e., arsenic concentrations) on a continuous scale. For example, the minimum detectable odds ratio for bladder cancer and the highest 2% of exposure is 1.75 based on a linear or quadratic model, with 80% power and an alpha of 0.05.

Summary

We are conducting an epidemiologic study to investigate the effects of drinking water exposure to arsenic on risk of nonmelanoma skin and bladder cancer in a U.S. population. To accomplish this, we have specifically established a system of identifying nonmelanoma occurrences in the general population and utilize the New Hampshire State Cancer Registry for bladder cancer. Using HR-ICP-MS and a hydride-generation procedure, we are able to reliably detect arsenic at concentrations present in over 99% of samples tested. We are also using a biologic measure of arsenic exposure (toenail clippings) that, based on our pilot work, is highly correlated with water concentrations. We plan to further evaluate the relation between water and toenail levels in our larger study and to assess the contribution of other sources of arsenic exposure such as tobacco smoking and occupation on toenail levels. Our analysis of household water samples will also help clarify the sources and mechanisms of arsenic presence in the drinking water supply. As part of the study, we are establishing a specimen bank of blood and tumor samples that can be used in future studies to evaluate potential susceptibility genes or tumor markers of arsenic exposure. Based on the projected sample size, we expect that our study will help fill important gaps in our knowledge regarding the relation between arsenic exposure and skin and bladder cancer risk in the United States.

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